REMARKS

Claims 1- 17 are in this application. Claim 2 has been amended as explained below. Claims 9-17 have been added. Claim 9 is a method for the treatment of septic shock conditions comprising administering to a subject in need thereof orally at specified time intervals a dosage of curcumin in the range of from 40 mg/kg to 60 mg/kg of body weight which is effective to prevent neutrophil infiltration from blood vessels to underlying tissues. Claim 10 has been added which a claim for a method for treating septic shock which includes monitoring the patient's symptoms. Claim 14 is a method for controlling neutrophil infiltration during inflammatory conditions by administering to the subject a pharmacologically effective dose of curcumin. Support for the new claims is found throughout the specification including on pages 5, 6, 9 and 10.

The Examiner rejected claim 2 under 35 USC 112, second paragraph. Applicants respectfully traverse this rejection.

The preamble of claim 2 has been amended to read "A method for monitoring septic shock conditions in an animal".

Therefore, it is respectfully requested that the rejection of claims 2-8 be withdrawn.

The Examiner has rejected claim 1 as being anticipated by Aggarwal (WO 97/09877) and claims 2-8 as being obvious over Aggarwal in combination with Meisner. Applicants respectfully traverse these rejections.

The Examiner states that Aggarwal teaches in claim 1 a method of inhibiting the activation of the NF-kB transcription factor in an animal in need of such treatment comprising the step of administering to said animal a pharmacologically effective dose of curcumin.

Septic shock is a condition arising upon severe infection with gram-negative bacteria. The lipopolysaccharide (LPS) that comprises the outer wall of the gram-negative bacteria activates various cells primarily macrophages, monocytes and other leukocytes. These activated cells release various mediators such as tumor necrosis factor (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, also nitrous oxide, superoxide anions and lipid mediators (Michie HR, et al., N. Engl. J. Med., 318:1481-1486, 1988). These mediators in turn lead to the activation of various transcription factors like AP1, C/EBT, NF-κB, etc. These endogeneous mediators act together and lead to several pathophysiological reactions including fever, leukopenia, thrombocytopenia, intravascular coagulation and leukocyte infiltration

in various organs that may ultimately lead to death. Thus, there is a systemic response to the invading pathogen, leading to septic shock (reviewed by Carlos J. et al., Immunol. Today, 18:329-334, 1997). It must be noted that septic shock is a condition resulting from a totality of various mediators like cell adhesion molecules viz. ICAM-1, VCAM-1 and E selectin, pro inflammatory cytokines like IL-1, TNF-α, NO, MIP-1α, CINC etc. whose expression in turn may be dysregulated in septic shock conditions affecting the regulation of the biochemical pathways and physiological mechanisms occurring at the *in vivo* level.

An important point to be noted is that Aggarwal et al., have shown that curcumin inhibits TNF-α induced activation of NF-κB. From the previous paragraph it should be noted that TNF-α is only one of the many mediators of septic shock. Also it is important to mention that NF-κB is only one of the many transcription factors activated in response to TNF-α or other mediators of septic shock. Aggarwal et al. (WO 97/09877) have only shown inhibition of TNF-α induced activation of NF-κB in a transformed cell line ML-1α cell line (leukemic cells) *in vitro* and have extrapolated it to the prevention of septic shock *in vivo*. An animal is a composition of various cell types and multiple biochemical pathways that operate together to determine the pathophysiological state. Interplay of these pathways controls the efficacy of the drug/compound in an organism as can be easily noted from the above discussion.

As explained in the instant application, LPS was administered to animals to induce symptoms of septic shock. LPS is at the top of the cascade of septic shock events, whereas TNF-α and NF-κB are only one of the many mediators in the lower layers of the regulatory cascade. The Examiner has mentioned that Aggarwal fails to teach the use of curcumin for prevention of neutrophil infiltration from the blood vessels to the underlying tissues. Neutrophil infiltration results from the cumulative effect of many mediators acting together and is a primary step leading to organ damage during inflammatory conditions. Thus studying the neutrophil infiltration is more representative of the conditions leading to septic shock rather than studying only one of the mediators of the process upstream in the pathway such as TNF-α or NFκB . Neutrophil infiltration covers the effect of all the mediators of septic shock. The applicants have devised an *in vivo* method for the prevention of neutrophil infiltration leading to prevention of septic shock. This is a novel and important part of the finding wherein the inventors provide evidence for the first time and demonstrate *in vivo* that curcumin prevents infiltration of neutrophils to the underlying tissues and subsequently prevents organ damage. This prevention of neutrophil infiltration can be used for the treatment of the various inflammatory diseases including septic shock where the infiltration of neutrophils is the primary cause of damage to the tissues.

It should also be mentioned that Aggarwal et al. speculate that prevention of TNFα induced effect is protective for treating septic shock. Meisner have shown protective effect of PDTC *in vivo* with elevated levels of TNFα Thus the two

reports of Aggarwal and Meisner contradict each other. The extrapolation of preventing the effect of TNFα to the treatment of septic shock conditions is being contradicted by the experimental evidence provided by Meisner et al.

It is also important to mention that the Examiner have mentioned that Aggarwal fail to provide any experimental evidence and fail to teach the use of curcumin in treating LPS induced septic shock as well as monitoring the activity of curcumin in said conditions by observing septic shock symptoms. On the contrary, the inventors have used an endotoxin shock model in which LPS is being administered to the animals to induce symptoms of septic shock. The inventors for the first time demonstrate the protective effect of curcumin on LPS induced septic shock conditions in vivo.

The Examiner has noted that Meisner discloses the scientific background that the activation of NFκ B transcription factor is implicated in the induction of numerous pro-inflammatory cytokines in septic shock conditions. However, the Examiner must also note that no experimental evidence has been provided for the same. In the literature, various molecules from natural and synthetic sources including sangunarine, N-acetylcysteine, glucocorticoids etc. have been shown to inhibit the activation of NF-κB in various cell types (J. Immunol. 1994, 153:2681 Chaturvedi et al J. Biol Chem 1997, 272 30129-30134, Brojstan et al, 1997 J Immuno. 1997: 3836-3844) It is also reported that inhibition of NF-κB is useful for the treatment of various inflammatory disorders including atherosclerosis, arthritis, tumor metastatis, diabetes (Chen et al. Clinical Chemistry, 1999, 45:7-17; Baeuerle and Henkel, Ann. Rev. Immunol, 1994 12: 141-179.

All NF-κB inhibitors cannot be used for the treatment of various inflammatory diseases including septic shock.

Accordingly, applicants submit that this application is in condition for allowance and favorable consideration is respectfully requested.

Respectfully submitted,

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2 (Twice Amended). A method for monitoring septic shock conditions in an animal wherein the method comprises:

a) injecting intraperitoneally a bacterial lipopolysaccharide (LPS) solution to an animal to induce septic shock,

b) administering orally a pharmacologically effective dose of curcumin prior to and after the injection of LPS,

c) observing every two to three hours reduction in severity of septic shock symptoms, the symptoms selected from shivering, lethargy, fever, watery eyes and diarrhea and monitoring the survival of the animal 8 hours after administering the LPS injection, and

d) furthering probing the reduction in neutrophil infiltration from blood vessels to the underlying tissue by staining and microscopic examination for checking the extent of inflammation.